AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-97 (canceled).

Claim 98 (Currently Amended): The method of claim [[63]] 119 wherein the first and second oligonucleotides [[used]] are selected from the group consisting of:

Seq Id 10: 5' – GGG GTA CTA CAG CGC CCT GA – 3'
Seq Id 19: 5'- GGG GTA CTA CAG CGC CCT GA –3'
|
FAM
Seq Id 13: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
Seq Id 20: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
|
JOE
Seq Id 12: 5' – ATG GCC ATC GTC CTG GAA GAT GGC CAT GG – 3'

Seq Id 22: 5' – DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG – 3'
|
JOE
Seq Id 23: 5'– DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG –3'
|
FAM
Seq Id 24: 5' – GCT CAT GGC GCC TGC CTG G – 3'
|
DABCYL

Seq Id 11: 5'- ATG GCC ATG TCC TGG AAG ATG GCC ATG G-3'

Seq Id -21: 5' – GGG GTA CTA CAG CGC CCT – 3' Seq Id -21

|
FAM

Seq Id 25: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
|
Rhod

Seq Id 26: 5'- GTC CTG GAA GAT GGC CAT GG – 3'
|
JOE

Seq Id 29: 5' GGC AAT GAA AAG CCA CTT CT – 3' as a forward primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome[[.]]; and

Seq Id 30: 5' TTA ACC GGC GAT TGA GTA CC – 3' as a reverse primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome.

Claim 99 (Previously Presented): A kit for use in method of analogous detection and/or quantitation of target nucleic acid sequence or sequences present in a sample comprising

a. a polymerase or polymerases
b. at least two oligonucleotides as a pair of primers for amplification of said target sequence such that after amplifiction the 3' ends of said pair of primers are on two opposite strands and separated from one another by 0-25 nucleotide pairs in the final amplification product;
c. deoxynucleotides in solution (water or buffer) or lyophilized
d. a reaction buffer for the nucleic acid amplification reaction.

Claim 100 (Currently Amended): The kit of claim 99 wherein the oligonucleotides as pair of primers are ~~suitably~~ labeled separately at or near ~~preferably near~~ their 3'ends with a donor or an acceptor MET/FRET moiety, and ~~contains~~ contain a free 3'hydroxy group for a polymerase to extend, the said donor and acceptor MET moieties belonging to a molecular energy transfer pair and so configured that the donor and the acceptor moieties come close within MET/FRET distance in the

amplification product and the nucleotides to which the donor and acceptor moieties are attached are 0-25 nucleotide pairs away.

Claim 101 (Currently Amended): The kit of claim 100 wherein at least the acceptor moiety of the acceptor MET moiety labeled oligonucleotide primer is provided quenched when the same is not incorporated into the amplification product or both the donor and acceptor MET moieties of the respectively labeled oligonucleotide primers are provided quenched when the same are not incorporated into the amplification product, the above quenched oligonucleotide primers being the oligonucleotide primers of claim [[69]] 124.

Claim 102 (Previously Presented): The kit of claim 99 wherein additionally positive control template and suitable MET/FRET labeled primers are also included as control for amplification reaction.

Claim 103 (Previously Presented): The kit of claim 99 wherein the first oligonucleotide is labeled near 3' end with a donor MET/FRET moiety and a double stranded DNA intercalating dye capable of absorbing energy or light emitted by the donor moiety and emitting energy or light is also provided.

Claim 104 (Previously Presented): The kit of claim 99 wherein the first oligonucleotide is labeled near 3' end with an acceptor MET/FRET moiety and a double stranded DNA intercalating dye capable of emitting energy or light on illumination is provided such that the acceptor moiety is capable of absorbing the energy or light emitted by the intercalating dye and emitting energy or light.

Claim 105 (Previously Presented): The kit of claim 99 comprising multiple oligonucleotide sets of claim for detection and/or/quantitation of multiple target sequences.

Claim 106 (Currently Amended): A kit for the detection of target nucleic acid sequences or sequences using the oligonucleotides used in the [[methods]] method of claim [[67]] 120 wherein the detection and /or quantitation of amplified target nucleic acid sequences is accomplished by

providing the first oligonucleotide primer being labeled at or near 5'end with a binding moiety preferably biotin, or magnetic particle or microsphere or a hapten or the like or attached to an anchor oligonucleotide directly or through a linker which can be respectively captured by streptavidin or magnet or centrifugation or anti-hapten antibody, capture oligonucleotide or the like and the second oligonucleotide primer being labeled with a signaling moiety like a fluorophore, rare earth metal chelate, biotin or a hapten, the hapten being detected utilizing antihapten antibody-enzyme conjugate, steptavidin- enzyme conjugate and enzyme substrate, and other conjugates or by using unlabeled second oligonucleotide primer and providing fluorescently labeled nucleotide in the reaction mixture in appropriate concentration.

Claim 107 (Currently Amended): A kit or kits for the detection of target nucleic acid sequences providing all or more components using the oligonucleotides for detection used in methods of claim [[67]] 120 wherein the detection and/ or quantitation of amplified target nucleic acid is accomplished by providing double stranded DNA binding fluorescent dye selected from the group consisting of eithidium bromide, CYBER TM GREEN I, pico green, acridine orange, thiazole orange Yo PRO- 1 and chromomycin A3 ~~but not excluding others~~.

Claim 108 (Currently amended): The kit of claim 99 wherein the oligonucleotides [[used]] are selected from the group consisting of:

Seq Id 10: 5' – GGG GTA CTA CAG CGC CCT GA – 3'
Seq Id 19: 5'- GGG GTA CTA CAG CGC CCT GA –3'
|
FAM
Seq Id 13: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
Seq Id 20: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
|
JOE
Seq Id 12: 5' – ATG GCC ATC GTC CTG GAA GAT GGC CAT GG – 3'

Seq Id 22: 5' – DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG – 3'

|
JOE

Seq Id 23: 5'– DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG –3'

|

FAM

Seq Id 24: 5' – GCT CAT GGC GCC TGC CTG G – 3'

|

DABCYL

Seq Id 11: 5'- ATG GCC ATG TCC TGG AAG ATG GCC ATG G-3'

Seq Id 21: 5' – GGG GTA CTA CAG CGC CCT – 3'

|

FAM

Seq Id 25: 5' – GTC CTG GAA GAT GGC CAT GG – 3'

|

Rhod

Seq Id 26: 5'- GTC CTG GAA GAT GGC CAT GG – 3'

|

JOE

Seq Id 29: 5' GGC AAT GAA AAG CCA CTT CT – 3' as a forward primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome, and

Seq Id 30: 5' TTA ACC GGC GAT TGA GTA CC – 3' as a reverse primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome.

Claim 109 (Currently Amended): A method of manufacture of a kit for use in a method of analogous detection and/or quantitation of target nucleic acid sequence or sequences present in a sample comprising

a. providing a polymerase or polymerases
b. providing a first oligonucleotide of sequence complementary to the nucleotide sequence flanking a target nucleotide sequence suitably labeled with a donor MET/FRET moiety at or near 3' end.

c. providing a second oligonucleotide of sequence at 5' end of the first nucleotide sequence complementary to the nucleotide sequence flanking the target nucleotide sequence or the segment of the target nucleotide sequence suitably labeled with an acceptor MET/FRET moiety at or near 3' end.

d. providing deoxy nucleotides in solution (water or buffer) or lyophilized.

e. providing a reaction buffer for the nucleic acid amplification reaction.

wherein the first and the second oligonucleotide sequences comprise the two primers (forward and reverse) of many nucleic acid amplification reactions and adapted to generate a detectable signal if the two oligonucleotides get incorporated into two opposite strands of amplified product and come in right proximity an the first and second oligonucleotides are any of the quenched oligonucleotide primers of claim [[63]] 121.

Claims 110-116 (Canceled).

Claim 117 (Previously Presented): The kit of claim 99, wherein the polymerases are a reverse transcriptase, a T7 RNA polymerase, and a DNA polymerase.

Claim 118 (Canceled).

Claim 119 (New): An improved method of detection and/or quantification of a nucleic acid target sequence by a nucleic acid amplification reaction comprising:

(i) a first oligonucleotide sufficiently complementary to hybridize to a first nucleic acid strand of a target sequence and to act as a nucleic acid amplification primer, and a second oligonucleotide sufficiently complementary to hybridize to the complement of the said first nucleic acid strand and to act as a nucleic acid amplification primer in an appropriate reaction mixture containing the said sample and a polymerase or polymerases, where the polymerase or polymerases are selected from DNA polymerase, reverse transcriptase, and RNA polymerase, wherein the said eaction mixture is subjected to an initial denaturation followed by repeated cycles of a combination of a denaturation step and at least a selective annealing step, which is further followed by an optional extension step,

(ii) the said first and the second oligonucleotide primers do not form any combination in absence of an amplification reaction, wherein the improvement comprises:

(ii) the presence of a target sequence in the sample being detected with improved sensitivity and specificity of the amplification product through the generation of a signal mainly on formation of a combination of the two primers and a combination of the two labels on the two separate primers on target amplification, and illuminating the amplification reaction mixture;

(iii) determining an additional specificity of the target detection by subjecting the amplified product to a melting temperature analysis.

Claim 120 (New): The method of claim 119, wherein the amplification product is of the size of a primer dimer, and the primers are 10-40 nucleotides long.

Claim 121 (New): The method of claim 120, wherein the amplification product is of the size, the length of the first oligonucleotide primer plus the length of the second oligonucleotide primer plus 0 – 25 base pairs; and in the said amplification product the 3' ends of the two oligonucleotide primers are separated by 0 – 25 base pairs.

Claim 122 (New): The method of claim 120 wherein the first oligonucleotide primer is labeled with a first label moiety through an internal base near the 3' end, the second oligonucleotide primer is provided unlabeled, further comprising a third oligonucleotide having a 5' end and a 3' end and sufficiently complementary to the first oligonucleotide primer to form a stable hybrid with the first oligonucleotide primer, the said third oligonucleotide is labeled with a second label moiety; and a combination of the first and the second label moieties, which are donor – acceptor FRET moieties, being formed in absence of an amplification reaction; the said third oligonucleotide is not a primer

and is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or a non-oligonucleotide organic linker;

the signal of the first label moiety is quenched by the second label moiety or the signal of the second label moiety is quenched by the first label moiety when the first and the second label moieties are in combination in absence of a target amplification, the said first and second oligonucleotide primers are 15 – 35 base long and the said third oligonucleotide is 5 – 30 bases long, and the second label moiety on the third oligonucleotide is provided at the 5' end or between the 3' and 5' ends through an internal base and the said first and second label moieties are separated by 0 – 25 base pairs in the combination of the two moieties in absence of an amplification,

wherein a signal is generated on displacement of the second label moiety from the combination of the first and second label moieties during the amplification reaction and illuminating the amplification reaction mixture with a light specific for the excitation of one of the two label moieties.

Claim 123 (New): The method of claim 121 wherein the first oligonucleotide primer is labelled with a first label moiety through an internal base near the 3' end, the second oligonucleotide primer is provided labeled with a second label moiety through an internal base near

the 3' end in the said reaction mixture containing the said sample, the said reaction mixture is subjected to an initial denaturation followed by repeated cycles of a combination of a denaturation step and at least a selective annealing step, which is

further followed by an optional extension step,

(i) the said first and second oligonucleotide primers, and the said first and second label moieties on two separate primers do not form a combination in absence of an amplification reaction;

(ii) the presence of a target sequence in a sample is detected with improved specificity through the generation of a sensitized FRET emission signal on formation of a combination of the two label moieties on the two separate

primers as a result of an amplification, and illuminating the amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety and the first and second

label moieties are respectively the donor and the acceptor moieties of a FRET pair; the specificity of target detection is further improved by subjecting the amplified product to a melting temperature analysis, wherein the first labelled primer is provided as a quenched primer or a dual labelled quenched primer, both the first and second labelled primers are provided as quenched primers or dual labelled quenched primers.

Claim 124 (New): The method of claim 123, wherein the first labelled oligonucleotide primer is provided quenched by providing a third oligonucleotide labeled with a third label moiety and sufficiently complementary to the said first oligonucleotide primer to form a stable hybrid with the first oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification; the said third oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or a non-oligonucleotide organic linker;

the said first and second oligonucleotide primers are 15 – 35 bases long and the said third oligonucleotide is 5 – 30 bases long, the third label moiety on the third oligonucleotide is provided at or between 5' and 3' ends through an internal base and the said first and third label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification reaction, and the said first and third label moieties are two members of a first FRET pair and the first and second label moieties are two members of a second FRET pair, where the two pairs are not same,

the third label moiety quenches the signal from the first moiety when it is in combination with the said first moiety in absence of a target amplification, a sensitized FRET emission signal being generated on amplification of the target sequence through formation of a combination of the first and second label moieties and displacement of the third moiety from the combination of the first and third moieties in the amplification product, and illuminating the amplification reaction mixture with a light specific for excitation of the first label moiety and measuring the emission of the second moiety, wherein the first moiety is a donor and the second moiety is an acceptor, or with a light specific for excitation of the second label moiety and measuring the emission of the first moiety, wherein the first moiety is an acceptor and the second moiety is a donor.

Claim 125 (New): The method of claim 120, wherein both the first and second labelled oligonucleotide primers are provided quenched by providing a third oligonucleotide sufficiently complementary to the first oligonucleotide primer is provided and labeled with a third label moiety to form a stable hybrid with the first oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification; the said third oligonucleotide is not a primer and

is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

and is also provided a fourth oligonucleotide sufficiently complementary to the second oligonucleotide primer and labeled with a fourth label moiety to form a stable hybrid with the second oligonucleotide primer, and a combination of the second and the fourth label moieties in absence of an amplification; the said fourth oligonucleotide is not a primer and is provided unlinked or provided linked to the 5' end of the said second oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

the said first and second oligonucleotide primers are 15 – 35 bases long and the said third and fourth oligonucleotides are 5 – 30 bases long, the third label moiety on the third oligonucleotide is provided at or between 5' and 3' ends through an internal base and the said first and third label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification reaction, the fourth label moiety on the fourth oligonucleotide is provided at or between 5' and 3' ends through an internal base and the said second and fourth label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification reaction,

and the said first and third label moieties being two members of a first FRET pair, the second and fourth label moieties being two members of a second FRET pair, and the first and second label moieties being two members of a third FRET pair where the three pairs are different,

the third label moiety quenches the signal from the first moiety when it is in combination with the first moiety, and the fourth label moiety blocks or quenches the signal from the second moiety when it is in combination with the said second moiety in absence of a target amplification,

and a sensitized FRET signal is generated on amplification of the target sequence through the formation of a combination of the first and second label moieties in the amplification product and displacement of the third moiety from the combination of the first and third moieties, and displacement of the fourth moiety from the combination of the second and fourth moieties, and illuminating the amplification reaction mixture with a light specific for excitation of the first label moiety and measuring the emission of the second moiety.

Claim 126 (New): A method of nucleic acid detection by nucleic acid amplification as in claim 119, wherein a first and a second oligonucleotide primers are provided to amplify a first portion of a target nucleic acid present in a sample,

a third and a fourth oligonucleotide primers to amplify a second portion of the said first portion in a nested manner, wherein the amplification product is of the size of a primer dimer, and the said first, second, third and fourth oligonucleotide primers are 15 – 35 bases long.

Claim 127 (New): The method of claim 126, wherein the first and the second oligonucleotide primers are provided unlabeled, the third oligonucleotide primer is provided labeled with a first label moiety through an internal base near the 3' end, the fourth oligonucleotide primer is provided labeled with a second label moiety through an internal base near the 3' end, the said first and second label moieties do not form a combination in absence of an amplification reaction, and the said first, second, third and fourth oligonucleotide primers are 15 – 35 bases long such that a sensitized FRET signal is generated on carrying out of an amplification of a target sequence through formation of a combination of the first and the second label moieties in the amplification product

corresponding to the said second portion and illuminating the amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety.

Claim 128 (New): The method of claim 127, comprising further providing a fifth oligonucleotide sufficiently complementary to the third oligonucleotide primer and labeled with a third label moiety to form a stable hybrid with the third oligonucleotide primer and a combination of first and third label moieties in absence of an amplification; the said fifth oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker; the said first, second, third and fourth oligonucleotide primers are 15 – 35 bases long and the said fifth oligonucleotide is 5 –30 bases long, and the third label moiety on the fifth oligonucleotide is provided at or between 5' and 3' ends through an internal base, the said first and third label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification, the third label moiety quenches the signal from the first moiety when it is in combination with the first moiety in absence of a target amplification, and a sensitized FRET signal being generated on amplification of a target sequence through the formation of a combination of the first and second label moieties in the amplification product and displacement of the third moiety from the combination of the first and third moieties, and illuminating the amplification reaction mixture with a light specific for excitation of the first label moiety and measuring the emission of the second moiety, wherein the first moiety is a donor and the second moiety is an acceptor, or with a light specific for excitation of the second

label moiety and measuring the emission of the first moiety, wherein the first moiety is an acceptor and the second moiety is a donor.

Claim 129 (New): The method of claim 127 comprising further providing a fifth oligonucleotide sufficiently complementary to the third oligonucleotide primer and labeled with a third label moiety to form a stable hybrid with the third oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification; the said fifth oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker,

a sixth oligonucleotide sufficiently complementary to the fourth oligonucleotide primer and labeled with a fourth label moiety to form a stable hybrid with fourth oligonucleotide primer, and a combination of the second and the fourth label moieties in absence of an amplification; the said sixth oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said fourth oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker; the said first, second, third and fourth oligonucleotide primers are 15 - 35 bases long and the said fifth and sixth oligonucleotides are 6 –30 bases long, the third and fourth label moieties on the fifth and sixth oligonucleotides respectively are provided at or

between 5' and 3' ends through internal bases and the said first and third label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification reaction, and the said second and fourth label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification reaction,where the third label moiety quenches the signal from the first moiety when it is in combination with the first moiety, and the fourth label moiety quenches the signal from the second moiety when it is in combination with the said second moiety in absence of an target amplification,

a sensitized FRET signal being generated on amplification of a target sequence by formation of a combination of the first and second label moieties in the amplification product and displacement of the third moiety from the combination of the first and third moieties, and displacement of the fourth moiety from the combination of the second and fourth moieties, and illuminating the

amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety.

Claim 130 (New): A method of nucleic acid detection by nucleic acid amplification as in claim 121 wherein a first and a second oligonucleotide primer are provided to amplify a first portion of a target nucleic acid present in a sample,

a third oligonucleotide primer is also provided to amplify in combination with the said first oligonucleotide primer a second portion of the above said first portion in a nested manner, the said second portion is amplified; or the said first and third oligonucleotide primers are the first and second oligonucleotide primers, and the said first, second, and third oligonucleotide primers are 15 – 35 bases long.

Claim 131 (New): The method of claim 130, wherein the first oligonucleotide primer is provided labeled with a first label moiety through an internal base near the 3' end, the second oligonucleotide primer is provided unlabeled and the third oligonucleotide primer is

provided labeled with a second label moiety through an internal base near the 3' end, the said first, second, and third oligonucleotide primers are 15 – 35 bases long,

a sensitized FRET signal being generated on amplification of a target sequence through formation of a combination of the said first and second label moieties in the amplification product generated by the said first and the third oligonucleotide primers, and illuminating the amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety.

Claim 132 (New): The method of claim 131, comprising further providing a fourth oligonucleotide sufficiently complementary to the first oligonucleotide primer and labeled with a third label moiety to form a stable hybrid with the first oligonucleotide primer, and a combination of the first and third label moieties in absence of an amplification; the said fourth oligonucleotide is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

alternatively, the fourth oligonucleotide is provided labeled with a third label moiety and is sufficiently complementary to the third oligonucleotide primer to form a stable hybrid with the third oligonucleotide primer and a combination of the second and the third label moiety in absence of an amplification, and is also provided unlinked, or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

the said first, second, and third oligonucleotide primers are 15 – 35 bases long and the fourth oligonucleotide is 5 – 30 bases long, the third label moiety on the fourth oligoucleotide is provided at or between 3' and 5' ends through an internal base, the said third label moiety is separated by 0 – 30 base pairs in the two separate

respective combinations of the first and the third moieties, or the second and the third moieties in absence of an amplification reaction, where the third label moiety blocks or quenches the signal from the first moiety when it is in combination with the first moiety in absence of an amplification; alternatively the third label moiety blocks or quenches the signal from the second moiety when it is in combination with the second label moiety in absence of a target amplification,

and a sensitized FRET signal is generated on amplification of the target sequence through formation of a combination of the said first and second label moieties in the amplification product and displacement of the third moiety from the combination of the first and third moieties, and in alternative case displacement of the third moiety from the combination of the second and third moieties, and illuminating the amplification reaction mixture with a light specific for excitation of the first label moiety and measuring the emission of the second moiety, such that the first moiety is a donor and the second moiety is an acceptor, or with a light specific for excitation of the second label moiety and measuring the emission of the first moiety such that the second moiety is a donor and the first moiety is an acceptor.

Claim 133 (New): The method of claim 131, further providing a fourth oligonucleotide labeled with a third label moiety and sufficiently complementary to the first oligonucleotide to form a stable hybrid with the first oligonucleotide, and a combination of the first and third label moieties; the said fourth oligonucleotide is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

a fifth oligonucleotide labeled with a fourth label moiety and sufficiently complementary to the third oligonucleotide to form a stable hybrid with the third oligonucleotide, and a combination of the second and fourth label moieties; the said fifth oligonucleotide is provided unlinked or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker; the said first, second, and third oligonucleotide primers are 15 – 35 bases long and the fourth and fifth oligonucleotides are 5 – 30 bases long, and the third and fourth label moieties on the fourth and fifth oligonucleotides are provided at or between 5' and 3' ends through internal bases and the said first and third label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification, and the said second and fourth label moieties are separated by 0 – 30 base pairs in the combination of the two moieties in absence of an amplification,where the third label moiety blocks the signal from the first label moiety when it is in combination with the said first moiety in absence of a target amplification, and the fourth label moiety blocks or quenches the signal from the second moiety when it is in combination with the said second moiety in absence of a target amplification, a sensitized FRET signal being generated on amplification of the target sequence through formation of a combination of the first and second label moieties and displacement of the third moiety from the combination of the first and third moieties, and displacement of the fourth moiety from the combination of the

second and fourth moieties in the amplification product, and illuminating the amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety.

Claim 134 (New): The method of claim 130 wherein the first oligonucleotide primer is provided unlabeled, the second oligonucleotide primer is provided unlabeled, and the third oligonucleotide is provided labeled with a first label moiety through an internal base near the 3' end and is also provided a fourth oligonucleotide labeled with a second label moiety and sufficiently complementary to the third oligonucleotide to form a stable hybrid with the third oligonucleotide primer and a combination of the first and second label moieties, in absence of an amplification;

said fourth oligonucleotide is provided unlinked, or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker;

the said first, second, and third oligonucleotide primers are 15 – 35 base long and the fourth oligonucleotide is 5 – 30 base long and the second label moiety is provided at 5' end or between 5' and 3' ends of the fourth oligonucleotide through an internal base, the said first and second label moieties are separated by 0 – 25 base pairs in the combination of the two moieties in absence of an amplification reaction;

a signal being generated on amplification of the target sequence through displacement of the second label moiety from the combination of the first and second label moieties during the amplification reaction and illuminating the amplification reaction mixture with a light specific for the excitation of one of the two label moieties and measuring the emission of the same moiety.

Claim 135 (New): A method of nucleic acid detection by nucleic acid amplification as in claim 119, providing a first oligonucleotide primer labeled with a first label moiety near the 3' end, and a second oligonucleotide primer labeled with a second label moiety near the 3' end, and is also provided a third oligonucleotide labeled with a third label moiety and sufficiently complementary to the first oligonucleotide primer to form a stable hybrid with the first oligonucleotide primer, and a combination of the first and third label moieties in absence of an amplification reaction; the said third oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said first oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker; one moiety of the combinations of the said first and third moieties quenches the signal of the other moiety when they are in a combination in absence of a target amplification, the said first and second oligonucleotide primers are 15 – 35 bases long and the third oligonucleotide is 5 – 30 bases long, and the third label moiety is provided at 5' or 3' end or between 3' and 5' ends of the third oligonucleotide through an internal base,

the said first and third label moieties are separated by 0 – 25 base pairs in the combination of the two moieties,and the first and the third label moieties are the members of a first FRET pair and the first and the second label moieties are the members of the same first FRET pair or of a second FRET pair and the first and second moieties are donor – acceptor or acceptor – donor moieties, either a sensitized FRET signal is generated on amplification of the target sequence through formation of a combination of the first and second label moieties and displacement of the third moiety from the combination of the first and third label moieties in the amplification product and illuminating the

reaction mixture with lights or radiations specific for the excitation of the first moiety and third moiety, and measuring the emission of the second moiety and third moiety respectively or similarly exciting the second moiety and third moiety and measuring the emission of the first moiety and third moiety respectively, where the first and third label moieties are the two members of a first FRET pair and the first and second label moieties are the two members of a second FRET pair, where the two pairs are not the same, or a signal is generated through formation of a combination of the first and second label moieties in the amplification product, where the bases to which the first and the second label moieties are attached are separated by a distance of 10 base pairs or more in the amplification product, such that the two label moieties are separated by a distance more than the distance of 50 % energy transfer of the two label moieties, and displacement of the third label moiety from the said combination of the first and the third moieties in the amplification product, such that the bases to which the first and the third moieties are attached are separated by a distance more than 20 base pairs in the amplification product, and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the first moiety or illuminating the reaction mixture with a light specific for the excitation of the third label moiety and measuring the emission of the third moiety;

wherein if a primer dimer is formed, the said first and the second label moieties form a combination and the bases to which the said first and second moieties are attached are separated by 0 – 25 base pairs such that the two moieties are within the distance of a maximum of 50% energy transfer of the two labeled moieties and a signal from such an amplification product is subtracted from the signal resulting in elimination of noise signal from the primer dimer.

Claim 136 (New): The method of claim 119, wherein a non-target sequence is attached to a target sequence by providing a polymerase or reverse transcriptase or a polymerase and a reverse transcriptase and a first oligonucleotide which contains a first sequence of length 10 – 30 bases sufficiently complementary to the target sequence at the 3' end so that it can prime a polymerase or a reverse transcriptase extension reaction, and a second sequence of length 15 – 30 bases at the 5' end of the said first sequence, where the second sequence is not complementary to the target sequence,

alternately a first oligonucleotide containing only the above second nontarget sequence of length 15 – 30 base which is non-complementary to the target sequence is ligated to a target

sequence or to a restricted target sequence, wherein a target sequence treated with a restriction enzyme or enzymes) in a ligation reaction,

and the said non-target second sequence is subjected to a nucleic acid amplification reaction by providing a second oligonucleotide which is a primer, and a third oligonucleotide which is also a primer, both the second and the third oligonucleotide primers are of length 15 – 30 bases,

the third oligonucleotide primer is either identical or sufficiently identical to the above said second non-target sequence of the first oligonucleotide appended to the target sequence to hybridize to the complement of the non-target second sequence appended target, and the second oligonucleotide primer is capable of hybridizing to the target sequence,wherein the said second oligonucleotide primer is provided labeled with a first label moiety through an internal base near the 3' end, and the third oligonucleotide primer is provided labeled with a second label moiety through an internal base near the 3' end;

a sensitized FRET signal is generated on amplification of the target sequence through formation of a combination of the first and second label moieties in the amplification product and illuminating the amplification reaction mixture with a light specific for the excitation of the first label moiety and measuring the emission of the second moiety.

Claim 137 (New): The method of claim 136, wherein the said second oligonucleotide primer is provided labeled with a first label moiety through an internal base near 3' end, the third oligonucleotide primer is provided labeled with a second label moiety through an internal base near 3' end, and further providing a fourth oligonucleotide labeled with a third label moiety and sufficiently complementary to the second oligonucleotide primer to form a stable hybrid with the second oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification, the third label moiety is provided at 5' end or between 3' and 5' ends of the fourth oligonucleotide and the said third label moiety is separated by 0 – 5 base pairs from the first moiety when they are in a combination in absence of an amplification reaction;

said fourth oligonucleotide not being a primer and is provided unlinked, or linked to the 5' end of the said second oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker, the said fourth oligonucleotide is 5 – 25 bases long

where the third label moiety quenches the signal from the first label moiety or the first label moiety quenches the signal from the third label moiety when the third moiety is in a combination with the first label moiety in absence of an amplification reaction, the said second and third moieties are same or different;

either a sensitized FRET signal being generated on amplification of the target sequence through formation of a combination of the first and second label moieties and displacement of the third moiety from the combination of the first and third label moieties in the amplification product and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the second moiety or exciting the second moiety and measuring the emission of the first moiety, and exciting the third moiety and measuring the emission of the third moiety, where the first and third label moieties are the two members of a first FRET pair and the first and second label moieties are the two members of a second FRET pair, where the two pairs are not same,

or a signal being generated on amplification of the target sequence by formation of a combination of the first and second label moieties in the amplification product, where the bases to which the first and the second label moieties are attached are separated by a distance of 10 base pairs or more in the amplification product, such that the two label moieties are separated by a distance more than the distance of 50 % energy transfer of the two label moieties, and displacement of the third moiety from the combination of the first and third label moieties in the amplification product, such that the bases to which the first and the third moieties are attached are separated by a distance more than 20 base pairs in the amplification product, and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the first moiety or exciting the third label moiety and measuring the emission of the third moiety, where the first and the third label moieties are the members of a first FRET pair, and the first and the second label moieties are the members of a second FRET pair and the two pairs being same or different; and if formation of a primer dimer occurs said first and the second label moieties form a combination and the bases to which the said first and second moieties are attached are separated by 0 – 25 base pairs and two moieties are within the distance of a maximum of 50% energy transfer of the two label moieties, and a signal from such an amplification product is subtracted from the signal resulting in elimination of noise signal from the primer dimer.

Claim 138 (New): The method of claim 136, wherein the said second oligonucleotide primer is provided labeled with a first label moiety through an internal base near 3' end, and the third oligonucleotide primer is provided labeled with a second label moiety through an internal base near 3' end, and further providing a fourth oligonucleotide labeled with a third label moiety and sufficiently complementary to the third oligonucleotide primer to form a stable hybrid with the third oligonucleotide primer, and a combination of the second and the third label moieties, the third label moiety is provided at 5' end or between 3' and 5' ends of the fourth oligonucleotide, the said third label moiety is separated by 0 – 25 base pairs from the second moiety when they are in a combination in absence of an amplification reaction;

said fourth oligonucleotide is not a primer and is provided unlinked, or provided linked to the 5' end of the said third oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker, wherein the third label moiety quenches the signal from the second label moiety or the second label moiety quenches the signal from the third label moiety when they are in a combination in absence of an amplification reaction, the said fourth oligonucleotide is 5 – 25 base long, the said first and third moieties are same or different, either a sensitized FRET signal is generated on amplification of the target sequence through formation of a combination of the first and second label moieties and displacement of the third moiety from the combination of the second and third label moieties in the amplification product and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the second moiety or exciting the second moiety and measuring the emission of the first moiety, and exciting the third moiety and measuring the emission of the third moiety, where the second and third label moieties are the two members of a first FRET pair and the first and the second label moieties are the members of a second FRET pair, where the two pairs are not same, or a signal is generated on amplification of the target sequence by formation of a combination of the first and second label moieties in the amplification product, where the bases to which the first and the second label moieties are attached are separated by a distance of at least 10 base pairs in the amplification product such that the two label moieties are separated by a distance more than the distance of 50 % energy transfer of the two label moieties, and displacement of the third moiety from the combination of the second and third label moieties in the amplification product and illuminating the reaction mixture with a light specific

for the excitation of the second moiety and measuring the emission of the second moiety or exciting the third label moiety and measuring the emission of the third moiety, where the second and the third label moieties are the members of a first FRET pair, and the first and the second label moieties are the members of a second FRET pair and the two pairs can be same or different; and if primer dimer forms, said first and the second label moieties form a combination and the bases to which the said first and second moieties are attached are separated by 0 – 25 base pairs and two moieties are within the distance of a maximum of 50% energy transfer of the two label moieties, and a signal from such an amplification product is subtracted from the signal resulting in elimination of noise signal from the primer dimer.

Claim 139 (New): A method of nucleic acid detection or quantitation by nucleic acid amplification wherein the second oligonucleotide primer of claim 137 provided unlabeled and the fourth oligonucleotide is provided labeled with a second label moiety, and the first and second label moieties are the two members of a FRET pair,

a signal being generated on amplification of a target sequence on displacement of the second moiety from the combination of the first and second label moieties and either illuminating the first moiety and measuring the emission of the first moiety or illuminating the second moiety and measuring the emission of the second moiety, where the first and second moieties block or quench emission of each other when they are in a combination in absence of target amplification.

Claim 140 (New): The method of claim 136, wherein the detection or quantitation of multiple target sequences by nucleic acid amplification is achieved by providing the labeled third oligonucleotide primer or the combination of the third oligonucleotide primer labeled with the second moiety and the labeled fourth oligonucleotide labeled with a third label moiety and sufficiently complementary to the third oligonucleotide primer to form a stable hybrid with the third oligonucleotide primer, and a combination of the second and the third label moieties in absence of an amplification reaction, unlinked or linked to the said third oligonucleotide primer through an oligonucleotide or a non-oligonucleotide organic linker or linker and spacer as a single common or universal primer for all the target sequences along with multiple individual second oligonucleotide primers for each target sequence.

Claim 141 (New): The method of claim 135, wherein multiple amplification primer pairs and other components are provided for amplification of a multiplicity of target sequences.

Claim 142 (New): The method of claim 135, wherein the first or second oligonucleotide primer is provided attached or fixed covalently through the 5' end or an internal nucleotide to a solid support through a linker or linker and spacer, and other amplification primers and reagents are provided in an aqueous phase in contact with the said solid phase, where the efficiency of amplification and detection of a target sequence in heterogeneous phase is improved by amplifying an amplification product of the size of primer dimer, the solid support to which the first oligonucleotide primer is attached is non-porous and transparent or translucent, glass, glass wafer, tubes or wells of a microtiter plate, or a plastic selected from the group consisting of: polystyrene, polyethylene, and polypropylene.

Claim 143 (New): The method of claim 136, wherein multiples of the said second oligonucleotide primer for amplification of multiple target sequences attached or fixed covalently through the 5' end or an internal nucleotide to a solid support through a linker or linker and spacer, and a common or universal third oligonucleotide primer common for all target sequences and other oligonucleotides and reagents are provided in an aqueous phase in contact with the said solid phase for the detection or quantitation of multiple target sequences in a sample; the said solid support to which the second oligonucleotide primers are attached is non-porous and transparent or translucent, glass, glass wafer tubes or wells of a microtiter plate, or a plastic selected from the group consisting of: polystyrene, polyethylene, and polypropylene.

Claim 144 (New): The method of claim 119, wherein two non-target sequences are attached on two sides of a target sequence by providing a polymerase or a polymerase and a reverse transcriptase or a ligase, a first oligonucleotide which contains at the 3' end a first sequence of length 10 – 30 bases sufficiently complementary to the target sequence so that it can hybridize to the target and prime a polymerase or a reverse transcriptase extension reaction, or a ligation reaction and a second sequence of length 15 – 30 bases at the 5' end of the said first sequence, where the second

sequence is a non-target sequence, and a second oligonucleotide which contains at the 3' end a third sequence of length 10 – 30 bases sufficiently complementary to the complement of the target sequence so that it can hybridize to the complement of the target and prime a polymerase or a reverse transcriptase extension reaction or a ligation reaction, and a fourth sequence of length 15 – 30 bases at the 5' end of the said first sequence, where the fourth sequence is a non-target sequence, alternately a first oligonucleotide containing only the above second non-target sequence of length 15 – 30 bases and a second oligonucleotide containing only the above fourth non-target sequence of length 15 – 30 bases which are non-complementary to the target sequence are ligated to a target sequence or to a target sequence digested with restriction enzymes in a ligation reaction,or in a combination of above primer extension and ligation reaction, and said second and fourth non-target sequence appended target sequence is subjected to a nucleic acid amplification reaction by providing a third oligonucleotide which is a primer, and a fourth oligonucleotide which is also a primer, both the third and fourth oligonucleotide primers are of length 15 – 30 bases, the said third oligonucleotide primer is either identical or sufficiently identical to the above said second on-target sequence of the first oligonucleotide appended to the target sequence such that it can hybridize to the complement of the above second sequence of non-target second sequence appended target, and the above said fourth oligonucleotide primer is sufficiently complementary to the above said fourth non-target sequence of the second oligonucleotide and hybridize to the above said fourth non-target sequence appended to the target sequence, the said third oligonucleotide primer is provided labeled with a first label moiety through an internal base near the 3' end, and the fourth oligonucleotide primer is provided labeled with a second label moiety through an internal base near the 3' end;

and is also provided a fifth oligonucleotide labeled with a third label moiety and sufficiently complementary to the third oligonucleotide primer to form a stable hybrid with the third oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification reaction; where either the third label moiety blocks or quenches the signal from the first label moiety or the first label moiety blocks or quenches the signal from the third label moiety when they are in combination in absence of an amplification reaction, the said fifth oligonucleotide is 5 – 25 bases long, and provided unlinked or linked to the third oligonucleotide primer through an oligonucleotide or a non-oligonucleotide organic linker, the third label moiety is provided at 5'end, or between 3' and 5' ends of the fifth oligonucleotide through an internal base, the said third label

moiety is separated by .0 – 25 base pairs from the first label moiety in their combination in absence of an amplification reaction, the said first and third moieties are same or different; a signal is generated on amplification of the target sequence by formation of a combination of the first and second label moieties in the amplification product, where the bases to which the first and the second label moieties are attached are separated by a distance more than 10 base pairs in the amplification product, such that the two label moieties are separated by a distance more than the distance of 50 % energy transfer of the two label moieties, and displacement of the third moiety from the combination of the first and third label moieties in the amplification product, such that the bases to which the first and the third moieties are attached are separated by a distance more than 20 base pairs in the amplification product, and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the first moiety or exciting the third label moiety and measuring the emission of the third moiety, where the first and the third label moieties are the members of a first FRET pair, and the first and the second label moieties are the members of a second FRET pair and the two pairs can be same or different; and if a primer dimer forms said first and the second label moieties form a combination and the bases to which the said first and second moieties are attached are separated by 0 – 25 base pairs, and a signal from such an amplification product is subtracted from the signal resulting in elimination of noise signal from the primer dime.

Claim 145 (New): The method of claim 119 wherein the label moiety is mentioned the label moiety is a Molecular Energy Transfer (MET)/Fluorescent Resonance Energy Transfer (FRET) (MET/FRET) moiety and wherever a combination of two label moieties is mentioned such combination is mainly a MET or FRET pair combination, one label moiety being a donor MET/FRET moiety, and the other label moiety being an acceptor MET/FRET moiety, and such a combination is formed only when the two moieties come close to each other within their MET/FRET distance, namely the distance of 50 % energy transfer between the two moieties; on illumination with its specific excitation radiation or light the donor moiety emits light or radiation, which is different from the light or radiation of illumination, and is absorbed by the acceptor moiety; the acceptor moiety in turn emits radiation or light which is characteristic of the acceptor moiety and is different from that of the donor moiety as well as the light of illumination, this emission from the acceptor moiety produced on illumination of the donor moiety is a sensitized emission; when the two donor

and acceptor moieties are in combination the signal emitted by the acceptor moiety can be measured while the donor emission signal remains quenched; wherein the displacement of one label moiety from a combination of the two label moieties which is a combination of a FRET pair, wherein the donor and acceptor moiety are separated by a distance of 0- 25 base pairs, and the displacement is a displacement or separation of the above said two label moieties from each other beyond a distance of at least 10-25 base pairs, which is the distance of 50 % energy transfer between many donor-acceptor MET/FRET pairs so that the acceptor FRET moiety can not absorb or block / quench the radiation or light emitted by the donor allowing light or radiation emission from the donor moiety and measurement of donor specific signal and the acceptor itself can't emit any radiation or light; further the acceptor moiety can be a radiative acceptor moiety, which emits a radiation or light, or a non-radiative acceptor moiety or a quencher, which does not emit a radiation or light, and when a signal is generated and measured the signal is substantial; whenever more than two FRET moieties are used different permitted combinations of suitable donor and acceptor moieties are used.

Claim 146 (New): The method of claim 145 wherein the donor and acceptor moieties are selected from any of the known donor – acceptor FRET pairs , wherein a FRET pair is a combination of a donor and an acceptor moiety such that the absorption spectra of the acceptor FRET moiety overlaps with at least 25% of the emission spectra of the donor FRET moiety, and the donor moiety is selected from the group including fluorescein and fluorescein derivatives, carboxyfluorescein (FAM), coumarin, 5-(2'amino ethyl) amino napthlene – 1 – sulfonic acid (EDANS), rhodamine, anthranilamide, Reactive Red-4, europium and terbium chelate derivatives, a combination of an organic moiety having a large extinction conefficient of absorportion and a fluorophore; and the said acceptor moiety is selected from the group including fluorescein, fluorescein derivatives, 2' 7' – dimethoxy 4'5'- dichloro-6-carboxyfluorescein (JOE), ethidium, texas red, eosin, nitrotyrosine, malachite green, pyrene butyrate, 2-{(E)-3-[1-(5-But-2-ynylcarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-propenyl}-1-ethyl-3,3-dimethyl-5-sulfinooxy-3H-indolium (Cy3) dyes, 2-{(1E,3E)-5-[1-(5-But-2-ynyl scarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-penta-1,3-dienyl}-1-ethyl-3,3-dimethyl-5-sulfinooxy-3H-indolium (Cy5) dyes, 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), DABCYL derivatives, rhodamine, rhodamine derivatives, 6-carboxy-X-rhodamine (ROX), N,N,N',N'-tetramethyl-6-

carboxyrhodamine (TAMRA), Sulfonyl chloride derivative of sulforhodamine (Texas Red), gold nano particle, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system), and the quencher is selected from the group consisting of DABCYL and its derivatives, rhodamine and its derivatives, gold nano particles, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system).

Claim 147 (New): The method of claim 119, wherein the oligonucleotide primers are provided unlabeled and a double stranded DNA binding fluorescent dye is also provided, which gives increased fluorescent on binding to the double stranded nucleic acid amplification product, and a signal is generated on amplification of a target sequence due to increased fluorescence on binding of the DNA binding dye to the amplification product, and the fluorescent DNA binding dye is selected from the group consisting of: ethidium bromide, SYBR Green (2-[2-{(3-Dimethylaminopropyl-propyl)-amino}-1-phenyl-1H-quinolin-(4E)-ylidenemethyl]-3-methyl benzothiazol-3-ium); Pico Green [2-[N-bis-(3-dimethylaminopropyl)-amino]-4-[2,3- dihydro-3-methyl(benzol-2-yl)-methylidene]-1-phenyl quinolinium; Acridine Orange (N,N,N',N'-Tetramethyl-acridine- 3,6,-diamine); Thiazole Orange (1-Methyl-4-[(3-methyl-2(3H)- benzothiazolylidene)-methyl] quinolinium p-tosylate); Yo-PRO- 1(Quinolinium,4-O(((3-methyl-2-(3H)benzoxyazolidene) methyl-1-O3-trimethylaminopropyl)-diiodide); and Chromomycin A3 (3B-O-(4-O-acetyl-2,6-dideoxy-3-(C-methyl-alpha-L-arabino- hexopyranosyl)-7-methylolivomycin D).

Claim 148 (New): The method of claim 123, wherein any one or both of the third and fourth primers are provided labeled with a donor or an acceptor FRET moiety, and also provided is a double-stranded DNA binding dye suitable to act as an acceptor or donor respectively for the FRET moiety separately or as part of the labeled primer, a signal is generated on amplification of a target sequence on formation of a combination of the FRET moiety and the DNA binding dye in the amplification product, the FRET moieties are a combination of a donor and an acceptor moiety such that the absorption spectra of the acceptor FRET moiety overlaps with at least 25% of the emission spectra of the donor FRET moiety, and the donor moiety is selected from the group consisting of fluorescein, fluorescein derivatives, carboxyfluorescein (FAM), coumarin, 5-(2'amino ethyl) amino naphthalene-1-sulfonic acid (EDANS), rhodamine, anthranilamide, Reactive Red-4, europium and

terbium chelate derivatives, a combination of an organic moiety having a large extinction conefficient of absorption and a fluorophore; and the said acceptor moiety is selected from the group consisting of fluoroscein, fluorescein derivatives, 2'7'-dimethoxy 4'5'-dichloro-6-carboxyfluorescein (JOE), ethidium, texas red, eosin, nitrotyrosine, malachite green, pyrene butyrate, 2-{(E)-3-[1-(5-But-2-ynylcarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihyroindol-(2E)-ylidene]-propenyl}-1-ethyl-3,3-dimethyl-5-sulfinoxy-3H-indolium (Cy3) dyes, 2-{(1E,3E)-5-[1-(5-But-2-ynyl scarbomoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-penta-1,3-dienyl}-1-ethyl-3,3-dimethyl-5-sulfinoxy-3H-indolium (Cy5) dyes, 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), DABCYL derivatives, rhodamine, rhodamine derivatives, 6-carboxy-X-rhodamine (ROX), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), sulfonyl chloride derivative of sulforhodamine (Texas Red), gold nano particle, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system), and the quencher is selected from the group consisting of DABCYL and its derivatives, rhodamine and its derivatives, gold nano particles, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system), and the DNA intercalating dyes are selected from the group consisting of: ethidium bromide, SYBR Green (2-[2-{(3-Dimethylaminopropyl-propyl)-amino}-1-phenyl-1H-quinolin-(4E)-ylidenemethyl]-3-methyl benzothiazol-3-ium); Pico Green [2-[N-bis-(3-dimethylaminopropyl)-amino]-4-[2,3-dihydro-3-methyl(benzol-2-yl)-methylidene]-1-phenyl quinolinium; Acridine Orange (N,N,N',N'-Tetramethyl-acridine-3,6,-diamine); Thiazole Orange (1-Methyl-4-[(3-methyl-2(3H)-benzothiazolylidene)-methyl] quinolinium p-tosylate); Yo-PRO-1(Quinolinium,4-O(((3-methyl-2-(3H)benzoxyazolidene) methyl-1-O3-trimethylaminopropyl)-diiodide); and Chromomycin A3 (3B-O-(4-O-acetyl-2,6-dideoxy-3-(C-methyl-alpha-L-arabino-hexopyranosyl)-7-methylolivomycin D), more specifically a fluorescein labeled primer and a double stranded DNA binding dye ethidium bromide is used where fluorescein act as a donor and ethidium bromide acts as an acceptor.

Claim 149 (New): The method of claim 119 wherein is provided a first oligonucleotide, a second oligonucleotide, a third oligonucleotide primer, and a fourth oligonucleotide primer at appropriate concentrations along with other reagents, where the first and the second oligonucleotides can be extended by a polymerase or can be ligated by a ligase enzyme;

the first oligonucleotide contains at the 3' end a first sequence sufficiently complementary to a single strand of a target sequence so that the first oligonucleotide can hybridize to the single stranded target sequence, and a second sequence of sufficient length at the 5' end of the first sequence, which is not sufficiently complementary to the single stranded target sequence, and is provided unlabeled;

the second oligonucleotide contains at the 5' end a third sequence sufficiently complementary to the same single strand target sequence so that the second oligonucleotide can hybridize to the single stranded target sequence, and a fourth sequence of sufficient length at the 3' end of the third sequence, which is not sufficiently complementary to the target sequence, and is provided unlabeled, so that the first and second oligonucleotides can be ligated with a ligase on hybrdization to the said single stranded target,

the third oligonucleotide primer is either identical or sufficiently identical to the above said second sequence of the first oligonucleotide to hybridize to the complement of the above second sequence of the first oligonucleotide and is provided labeled with a first label moiety near the 3' end,

and the fourth oligonucleotide primer is sufficiently complementary to the above said fourth sequence of the second oligonucleotide to hybridize to the above fourth sequence of the second oligonucleotide and is provided labeled with a second label moiety near the 3' end,

and is also provided a fifth oligonucleotide labeled with a third label moiety and sufficiently complementary to the third oligonucleotide primer to form a stable hybrid with the third oligonucleotide primer, and a combination of the first and the third label moieties in absence of an amplification reaction;

where either the third label moiety blocks or quenches the signal from the first label moiety or the first label moiety blocks or quenches the signal from the third label moiety when they are in a combination in absence of an amplification reaction, the said fifth oligonucleotide is 5 – 30 bases long, and provided unlinked or linked to the third oligonucleotide primer through an oligonucleotide or a non-oligonucleotide organic linker, and the third label moiety is provided at 5' end or between 3' and 5' ends of the fifth oligonucleotide through an internal base, the said third label moiety is separated by 0 – 25 base pairs from the first label moiety in their combination in absence of an amplification reaction where the said first and third moieties are same or different;

the said third and fourth oligonucleotide primers are 15 – 35 bases long each and the first and the third sequences of the said first and second oligonucleotides are 10 – 30 bases long and the second and fourth sequences of the first and second oligonucleotides respectively are 15 – 35 bases long;

a signal is generated on amplification of the target sequence by formation of a combination of the first and second label moieties in the amplification product, where the bases to which the first and the second label moieties are attached are separated by a distance of at least 10 base pairs in the amplification product, wherein the two label moieties are separated by a distance more than the distance of 50 % energy transfer of the two label moieties, and displacement of the third moiety from the combination of the first and the third label moieties in the amplification product and illuminating the reaction mixture with a light specific for the excitation of the first moiety and measuring the emission of the first moiety or illuminating the reaction mixture with a light specific for the excitation of the third moiety and measuring the emission of the third moiety, where the first and the second label moieties are the members of a first FRET pair and the first and the third label moieties are the members of the same first FRET pair or of a second FRET pair, where the second and third moieties can be same or different,

in case of formation of a primer dimer the said first and the second label moities form a combination and the bases to which the said first and second moieties are attached are separated by 0 – 25 base pairs such that the two moieties are within the distance of a maximum of 50% energy transfer of the two label moieties, and a signal from such an amplification product is subtracted from the signal resulting in elimination of noise signal from the primer dimer.

Claim 150 (New): The method of claim 123, wherein any one or both of the first and second primers are provided labeled with a donor or an acceptor FRET moiety and is also provided at least one of the four deoxynucleotides labeled with a suitable acceptor or donor FRET moiety for the above donor or acceptor moieties respectively, and a FRET signal is generated on incorporation of the labeled nucleotide in amplification product in case of target amplification; the said donor and acceptor FRET moieties are such that the absorption spectra of the acceptor FRET moiety overlaps with at least 25% of the emission spectra of the donor FRET moiety and the donor moiety is selected from the group consisting of fluorescein, fluorescein derivatives, carboxyfluorescein (FAM),

coumarin, 5-(2'amino ethyl) amino naphthalene-1-sulfonic acid (EDANS), rhodamine, anthranilamide, Reactive Red-4, europium and terbium chelate derivatives, a combination of an organic moiety having a large extinction conefficient of absorption and a fluorophore; and the said acceptor moiety is selected from the group consisting of fluoroscein, fluorescein derivatives, 2'7'-dimethoxy 4'5'-dichloro-6-carboxyfluorescein (JOE), ethidium, texas red, eosin, nitrotyrosine, malachite green, pyrene butyrate, 2-{(E)-3-[1-(5-But-2-ynylcarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihyroindol-(2E)-ylidene]-propenyl}-1-ethyl-3,3-dimethyl-5-sulfinoxy-3H-indolium (Cy3) dyes, 2-{(1E,3E)-5-[1-(5-But-2-ynyl scarbomoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-penta-1,3-dienyl}-1-ethyl-3,3-dimethyl-5-sulfinoxy-3H-indolium (Cy5) dyes, 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), DABCYL derivatives, rhodamine, rhodamine derivatives, 6-carboxy-X-rhodamine (ROX), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), sulfonyl chloride derivative of sulforhodamine (Texas Red), gold nano particle, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system), and the quencher is selected from the group consisting of DABCYL and its derivatives, rhodamine and its derivatives, gold nano particles, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system).

Claim 151 (New): A method for the detection and/or quantitation of amplified target nucleic acid sequence as in claim 119, wherein a first oligonucleotide primer, and a second oligonucleotide primer are provided such that the first oligonucleotide primer is provided with a label moiety, which is a binding moiety selected from the group including biotin, magnetic particle, microsphrere, a hapten, an anchor oligonucleotide directly or through a linker, the said binding moiety can respectively be captured by any one of the capturing moieties streptavidin or magnet or centrifugation or an anti-hapten antibody, a capture oligonucleotide, the second oligonucleotide primer being labeled with another label moiety which is a signaling moiety including a fluorophore or a fluorescent dye, a rare earth metal chelate, biotin, gold nanoparticle or a hapten; alternatively either the second oligonucleotide primer is provided unlabeled or both the primers are provided unlabeled and are also provided in the reaction mixture a fluorescent dye, or a hapten or a fluorophore labeled nucleotide or nucleotides, and separately an anti-hapten antibody or stemptavidin-enzyme or gold conjugate and suitable substrate for signal generation;

and is also provided a suitable capture moiety wherein the capture moiety can capture the amplification product directly or through binding to the binding moiety, a suitable signal is generated from the above combinations; and the above said first and second oligonucleotide primers are first and second primers of claim 119 and are of length 15-35 nucleotides.

Claim 152 (New): The method of claim 119 wherein said nucleic acid amplification reaction comprise any known nucleic acid amplification reactions including polymerase chain reaction comprising the steps of adding a polymerase or polymerases, reaction buffer, deoxynucleoside triphosphates in addition to the effective amounts of amplification primers and other oligonucleotides and reagents to the sample, carrying out an initial denaturation followed by repeated cycles of a denaturation step and a selective annealing step, or repeated cycles of a denaturation step, a selective annealing step and an extension step, and an optional final extension step, exciting the reaction mixture with a donor exciting radiation or light, measuring the emission of an acceptor FRET moiety, or that of the donor.

Claim 153 (New): The method of claim 119, wherein said oligonucleotides are of linear or hairpin conformation and are selected from the group comprising DNA, RNA orchimeric mixtures, derivatives or modified versions thereof adapted for hybridizing and priming nucleic acid amplification reaction, and are deoxy oligonucleotides, oligonucleotide or peptide or locked nucleic acid or modified oligonucleotides; the target nucleic acid sequence is selected from genomic DNA, mRNA, RNAs, cDNA, amplification product, chemically or biochemically synthesized DNA or RNA.

Claim 154 (New): The method of claim 119 wherein the nucleic acid amplifications are a polymerase chain reaction (PCR), or a reverse transcription PCR (RT-PCR), or an allele specific PCR, or a methylation status PCR, or an in situ PCR, or a Triamplification, or a Nucleic acid sequence based amplification or an isothermal amplification, or Strand displacement amplification, or an immuno PCR.

Claim 155 (New): The method of claim 119, wherein the target nucleic acid sequence is an amplification product or the sequence of an infectious disease agent; or a genomic sequence of a human, animal, plant or any other living organism, a mutation in which is implicated to the presence of a disorder or disease; or a human, animal or plant genomic sequence, the presence or absence of which is implicated to a disorder or disease; or a human, animal or plant genomic sequence, the presence or absence of which is implicated to susceptibility to an infectious agent; or a human, animal, plant or any living organism genomic sequence the presence or absence of which is implicated to a genetic trait or genotyping of human, animal, plant, or the living organism; or a genomic sequence of an infectious agent, the presence or absence of which is implicated to strain typing; or a sequence of a gene a mutation of which is related to a particular allele of the gene.

Claim 156 (New): A kit for detection of a target nucleic acid sequence present in a sample by nucleic acid amplification which comprises:

a) a polymerase or polymerases;

b) a first oligonucleotide primer that can prime a nucleic acid amplification reaction and is labeled through an internal base near 3'end with a first label moiety capable of generating a signal; a second oligonucleotide primer that can prime a nucleic acid amplification reaction and is labeled through an internal base near 3' end with a second label moiety capable of generating a signal; a third oligonucleotide, which is not a primer and is provided unlinked,or provided linked to the said first oligonucleotide primer through an oligonucleotide or non-oligonucleotide organic linker, and the third oligonucleotide is also provided labeled at the 5' or 3' end or an internal base with a third label moiety, the said third oligonucleotide is sufficiently complementary to the said first oligonucleotide primer to form a stable hybrid with the first oligonucleotide primer and a combination of the first and third label moieties in absence of an amplification reaction; the said oligonucleotide primers are 15 – 35 bases long and the third oligonucleotide is 5 – 30 bases long; the said first, second and third label moieties are FRET moieties, and are so selected that the first and the second moieties are the members of a first FRET donor – acceptor pair, and the first and the third moieties are the members of a second or the same first FRET donor – acceptor pair, where the donor or donor moieties are fluorophores and the acceptor or acceptor moieties are fluorophores or non-fluorophores ;

c) deoxynucleotides or their mixture in a solution (water or buffer) or lyophilized;

d) reaction buffers; and

e) a sensitized FRET signal is generated on amplification of the target sequence by formation of a combination of the first and second llabel moieties and displacement of the third moiety from the combination of the first and third label moieties in the amplification product.

Claim 157 (New): The method of claim 126, wherein the oligonucleotide primers are provided unlabeled and also provided is a double stranded DNA binding fluorescent dye, which gives increased fluorescent on binding to the double stranded nucleic acid amplification product, and a signal is generated on amplification of a target sequence due to increased fluorescence on binding of the DNA binding dye to the amplification product, and the fluorescent DNA binding dye is selected from the group consisting of: ethidium bromide, SYBR Green (2-[2-{(3-Dimethylaminopropyl-propyl)-amino}-1-phenyl-1H-quinolin-(4E)-ylidenemethyl]-3-methyl benzothiazol-3-ium); Pico Green [2-[N-bis-(3-dimethylaminopropyl)-amino]-4-[2,3-dihydro-3-methyl(benzol-2-yl)-methylidene]-1-phenyl quinolinium; Acridine Orange (N,N,N',N'-Tetramethyl-acridine-3,6,-diamine); Thiazole Orange (1-Methyl-4-[(3-methyl-2(3H)-benzothiazolylidene)-methyl] quinolinium p-tosylate); Yo-PRO-1(Quinolinium,4-O(((3-methyl-2-(3H)benzoxyazolidene) methyl-1-O3-trimethylaminopropyl)-diiodide); and Chromomycin A3 (3B-O-(4-O-acetyl-2,6-dideoxy-3-(C-methyl-alpha-L-arabino-hexopyranosyl)-7-methylolivomycin D).

Claim 158 (New): The method of claim 123, wherein for the heterozygous mutation detection which comprises two amplification primer oligonucleotides, one labeled with a donor MET moiety near 3' end and the other being labeled with an acceptor MET moiety near 3' end, wherein a target amplification reaction and a thermal denaturation analysis of the amplification product or products thus amplified is carried out and in the same method, the labeled oligonucleotide primers are also provided in dual labeled quenched primer configuration.